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Revival of *Philozoon* Geddes for host-specialized dinoflagellates, ‘zooxanthellae’, in animals from coastal temperate zones of northern and southern hemispheres

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ABSTRACT

The dinoflagellate family Symbiodiniaceae comprises numerous genera and species with large differences in diversity, ecology and geographic distribution. An evolutionarily divergent lineage common in temperate symbiotic cnidarians and designated in the literature by several informal names including ‘temperate-A’, A₁, Phylotype A’ (A-prime) and ‘Mediterranean A’, is here assigned to the genus *Philozoon*. This genus was proposed by Geddes (1882) in one of the earliest papers that recognized ‘yellow cells’ as distinct biological entities separate from their animal and protist hosts. Using phylogenetic data from nuclear (rDNA), chloroplast (cp23S) and mitochondrial genes (*cob* and *cox1*), as well as morphology (cell size), ecological traits (host affinity) and geographic distributions, we emend the genus *Philozoon* Geddes and two of its species, *P. medusarum* and *P. actiniarum*, and describe six new species. Each symbiont species exhibits high host fidelity for particular species of sea anemone, soft coral, stony coral and a rhizostome jellyfish. *Philozoon* is most closely related to *Symbiodinium* (formerly Clade A), but, unlike its tropical counterpart, occurs in hosts in shallow temperate marine habitats in northern and southern hemispheres including the Mediterranean Sea, north-eastern Atlantic Ocean, eastern Australia, New Zealand and Chile. The existence of a species-diverse lineage adapted to cnidarian hosts living in high latitude habitats with inherently wide fluctuations in temperature calls further attention to the ecological and biogeographic reach of the Symbiodiniaceae.

HIGHLIGHTS

- A dinoflagellate genus symbiotic with temperate invertebrates is characterized and named using a discarded taxonomic term revived from the golden age of Natural Historians.
- The work highlights how animal–algal mutualisms are evolved to thrive under a broad range of environmental conditions.

KEYWORDS Adaptive radiation; ecological specialization; Mediterranean Sea; Symbiodiniaceae; temperate zone; zooxanthellae

Introduction

Dinoflagellate endosymbionts are essential to the survival and proliferation of numerous marine invertebrates. While abundant in shallow warm seas where their host corals build reef ecosystems, these mutualisms are widespread and found in many other marine settings. Knowledge of these symbioses began in the late 1800s, when natural historians correctly deduced that the small pigmented cells found in invertebrate animals and shell-bearing protists were distinct entities (Brandt, 1881; Geddes, 1882a). Karl Brandt (1881) published the first detailed papers to formally describe the yellow cells found in protists (Radiolaria) and in animals. In these, he established the genus

Zooxanthella K.Brandt and coined the term ‘zooxanthellae’ (Krueger, 2017). The experiments of Patrick Geddes on these ‘yellow’ cells supported Brandt’s findings, included characterization of their cell wall composition and demonstrated how animals with them were photosynthetic when exposed to light (Geddes, 1882b).

Geddes’s papers also proposed the genus name *Philozoon* Geddes and designated four species because they differed slightly in size, colour, mode of division and ‘behavior with reagents’ (presumably this related to differences in their biochemistry). He did so, initially unaware that it conflicted with *Zooxanthella* described by Brandt one year prior (Geddes, 1882a). Moreover, unlike

Brandt (1881), he provided no descriptions, or drawings, showing exactly how each of these ‘yellow cells’ differed from each other, nor were specimens from his work in Naples, Italy, preserved and archived. Months after recognizing the redundancy of *Philozoon* due to publication of *Zooxanthella* he wrote, ‘the attempt which has been made independently by Dr. Brandt and myself, at the generic and specific definition of the yellow cells . . . is doubtless premature, and I think it preferable to with-hold the present diagnosis of species’ (Geddes, 1882a: 394). Geddes therefore chose not to further his species descriptions and would soon transition from the biological sciences to an eminent career pioneering the profession of urban design and planning (Stalley, 1972; see Discussion).

For many reasons, *Philozoon* as a genus name was never adopted by subsequent investigators, although many decades later it was lectotypified by Loeblich & Loeblich (1966), who chose *P. medusarum* from the jellyfish *Cotylorhiza tuberculata* Macri (= *Cassiopeia borbonica*) as the type species. While some have argued rationally that *Philozoon* is an improper taxonomic term for reasons mentioned above (Blank & Trench, 1986; Yuasa *et al.*, 2016), it is considered validly published by the Index Nominum Algarum (ucjeps.berkeley.edu/ina) and *AlgaeBase* (Guiry & Guiry, 2020). Consequently, there is a need to resolve the identity and systematic placement of this genus and its species.

Molecular genetic evidence has substantially improved the taxonomic and systematic sorting of eukaryotic microbes (Adl *et al.*, 2019; Bik *et al.*, 2012; Leliaert *et al.* 2014). For symbiotic dinoflagellates, phylogenetic data continue to enhance our understanding of heredity and evolutionary history as new species are resolved and grouped into hierarchical taxonomic ranks with increased confidence (Price & Bhattacharya, 2017). More than a century after their discovery, the true identities of these morphologically cryptic symbionts are finally being properly characterized (Rowan & Powers, 1991; Gast & Caron, 1996; Siano *et al.*, 2010; Gottschling & McLean, 2013; Probert *et al.*, 2014; Yuasa *et al.*, 2016; LaJeunesse *et al.*, 2018). Presently, ‘zooxanthellae’ are a paraphyletic group represented by three dinophycean orders comprising numerous genera and a multitude of species (LaJeunesse *et al.*, 2018).

Dinoflagellates symbiotic with radiolarians in the genera *Collozoum*, *Thalassiocolla* and *Spongostaurus*, as well as those associated with the cnidarian chondrophore *Vellella vellella* are members of the genus *Zooxanthella* in the order Peridiniales (Gast & Caron, 1996; Gottschling & McLean, 2013; Probert *et al.*, 2014). The widespread endosymbionts of many invertebrates including reef corals, sea anemones, jellyfish, flatworms, giant clams and protists, including benthic foraminifera (Soritinae) and ciliates, are

in the family Symbiodiniaceae, order Suessiales (LaJeunesse, 2020). The family Symbiodiniaceae presently contains nine genera, as well as several phylogenetically divergent unnamed genus-level clades (LaJeunesse *et al.*, 2018; Nitschke *et al.*, 2020). These ‘stray’ lineages presently lack formally described species to serve as designated ‘type species’, required when erecting new genera.

Among the remaining symbiodiniacean lineages in need of a genus name, one is mutualistic with many cnidarians, including sea anemones (Actiniaria), stony corals (Scleractinia), gorgonians (Alcyonacea) and jellyfish (Scyphozoa). While common to host animals living in the Mediterranean Sea and Eastern North Atlantic (Savage *et al.*, 2002; Visram *et al.*, 2006; Casado-Amezúa *et al.*, 2014), recently a member of this group was identified in the sea anemone *Anthopleura aureoradiata* along the coast of New Zealand, indicating that its geographic distribution encompasses temperate zones of both northern and southern hemispheres (Howe, 2013). This lineage has been referred to in the literature as temperate-A (Savage *et al.*, 2002; Visram *et al.*, 2006), A’ (A-prime) (Barbrook *et al.*, 2006), A_I (Hansen & Daugbjerg, 2009), A1_Med & A1_NAtl (Grajales *et al.*, 2016) or ‘Mediterranean A’ (Hunter *et al.*, 2007), because it is most closely related to *Symbiodinium* (formerly clade A). Small changes in nucleotide sequences distinguish symbionts obtained from different host taxa, suggesting that multiple species comprise this group (Casado-Amezúa *et al.*, 2014).

Of particular significance, and pertaining to the work of Geddes, is that the rhizostome jellyfish, *Cotylorhiza tuberculata*, and the sea anemone, *Anemonia viridis*, are known to associate with a ‘temperate-A’ symbiont (Visram *et al.*, 2006; Mordret & D’Ambra, unpubl.). These are two host species whose symbionts Geddes studied and named, including the source of the generitype species for *Philozoon*. Here, our systematic revision assigns the evolutionarily divergent group of temperate symbiotic dinoflagellates to the genus proposed by Geddes and emends the original description of *Philozoon*. Moreover, genetic, morphological (cell size) and ecological data are compiled to emend species descriptions originally proposed by Geddes, as well as to describe six additional species found in host cnidarians from northern and southern temperate marine coastal ecosystems. The paper concludes by acknowledging the pioneering contributions of Patrick Geddes to the study of symbiotic mutualisms.

Materials and methods

Field collected samples

Animal specimens containing ‘temperate A’ Symbiodiniaceae were obtained from the North Sea,

Mediterranean Sea, New Zealand, Australia and Chile (Figs 1–5; Supplemental table S1). Fresh fragments of host tissue or entire polyps were placed in vials containing DMSO-EDTA-NaCl preservation buffer (Seutin *et al.*, 1991) and stored either at 4°C or at –20°C. Neotype and holotype specimens were deposited in the Algal Collection of the National Herbarium, Smithsonian Institution, Washington, DC, USA as US 227762.

Cell size measurements

Squash mounts of host tissue were prepared on a glass slide to image symbiont cells. The cells were visualized with Differential Interference Contrast microscopy on the Olympus Bx51 microscope (Olympus Corp., Tokyo, Japan) and images captured using an ORCA ER (Model C4742-80) and Olympus DP71 Hamamatsu digital camera (Olympus Corp., Tokyo, Japan) at Penn State University Microscopy Facility, University Park, PA. Length and width measurements using ImageJ 1.5w (Schneider *et al.*, 2012) were obtained from 75–200 individual ovate cells. The mean cell size was calculated along with standard deviation and 95% confidence intervals using Excel (Microsoft).

Genetic analyses

DNA extractions for most samples followed the protocol described by LaJeunesse *et al.* (2003). The partial chloroplast cp23S and mitochondrial *cob* and *cox* genes were amplified and directly sequenced for all cultures and field collected samples according to Zhang *et al.* (2000) and Zhang *et al.* (2008), respectively. The *psbA* non-coding region (*psbA^{ncr}*), a functional non-coding DNA region from the chloroplast mini-circle genes of dinoflagellates (Moore *et al.*, 2003; LaJeunesse & Thornhill, 2011), was also amplified and directly sequenced. The primers *psbAFor_1* (5′-GCA GCT CAT GGT TAT TTT GGT AGA C-3′) and *psbARev_1* (5′-AAT TCC CAT TCT CTA CCC ATC C-3′), designed to have efficacy on most Symbiodiniaceae, were used to amplify and sequence the non-coding region of the *psbA* minicircle (*psbA^{ncr}*) (LaJeunesse & Thornhill, 2011). The PCR conditions for amplification of the *psbA^{ncr}* were: 94°C for 2 min, then 40 cycles of 94°C for 10 s, 55°C for 30 s and 72°C for 2 min, followed by a final extension at 72°C for 10 min. Primers designed specifically to amplify the *psbA^{ncr}* of ‘temperate A’ symbionts are *Philozoon-psbAF* (5′-ATT TGG TTC ACA GCG CTT GG-3′) and *Philozoon-psbAR* (5′-CCA TTT GAC TCC CAC ACT GGA-3′). An annealing temperature of 57°C is optimal when using these primers in a PCR. Additional internal primers developed and used to sequence the *psbA^{ncr}* for the symbionts associated with the sea anemone *Cereus pedunculatus* are *Cereus-psb*

A-for (5′-CTC AAA AAG CCG AAC CCT GC-3′) and *Cereus-psbA-rev* (5′-AAG TAG ACT TGC CGG CTC AC-3′). Direct Sanger sequencing on PCR amplified DNA was performed using Big Dye 3.1 reagents (Life Sciences) and the reaction products analysed on an Applied Biosystems 3730XL instrument.

Base calling on chromatograms was visually inspected for accuracy (Geneious v. 11.0.3) and the edited sequences aligned initially using the online application of ClustalW2 (<http://www.ebi.ac.uk/Tools/msa/clustalw2/>). Further adjustments to alignments were made upon visual inspection of the output file. Final edited sequences were deposited in GenBank. Phylogenetic analyses using Maximum parsimony, confirmed with Maximum likelihood, were conducted using the software PAUP (v. 4.0a136; Swofford, 2014) on aligned sequences. Bootstrap support of branching nodes was based on 1000 replicates. A phylogenetic reconstruction of the *psbA^{ncr}* was conducted using Bayesian inference using MrBayes v3.2.1 (Ronquist *et al.*, 2012) implementing General Time Reversible (GTR)+Γ. Each MCMC analysis was run for 1.0×10^6 generations and sampled every 100 generations. The first 25% of trees were discarded as burn-in corresponding with the convergence of chains. Nexus files, one that contains the alignment of concatenated LSU, cp23S, *cob* and *cox1* sequences corresponding to species of *Philozoon* and *Symbiodinium*, and one that contains the alignment of *psbA^{ncr}* sequences representative of each *Philozoon* spp., are available in the Supplementary data.

Preparation of holotypes and neotypes

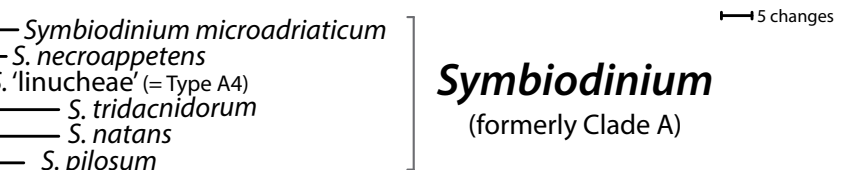
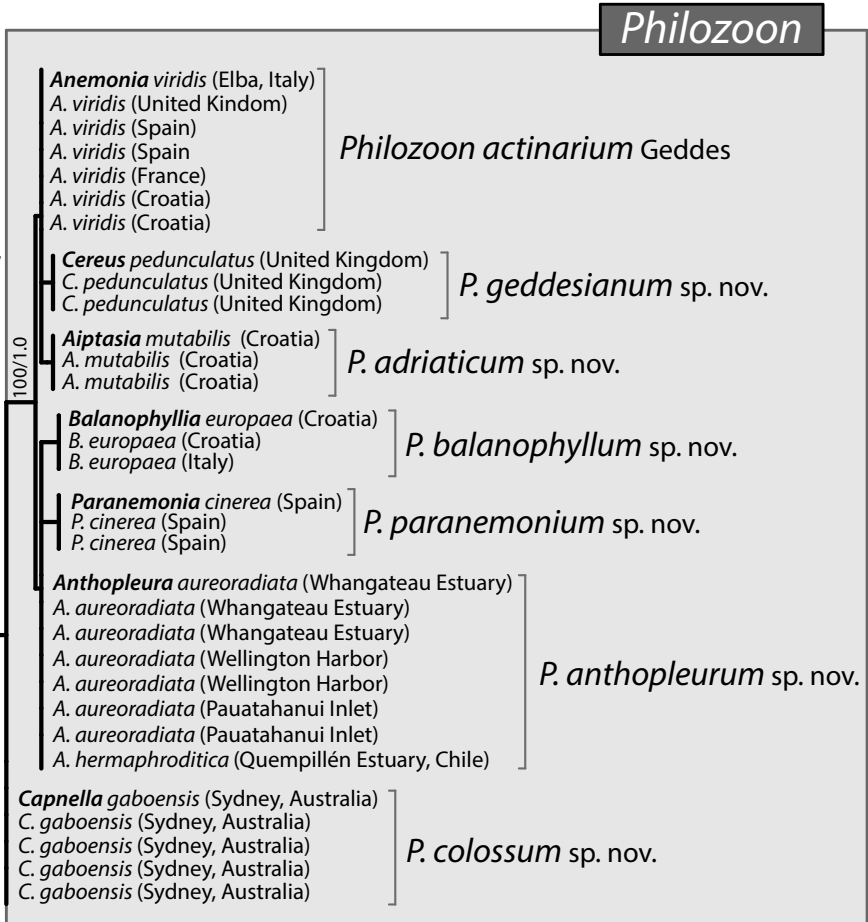
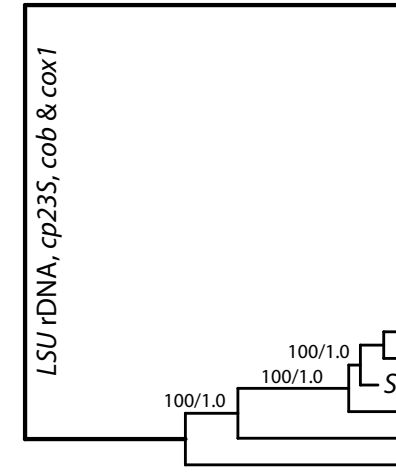
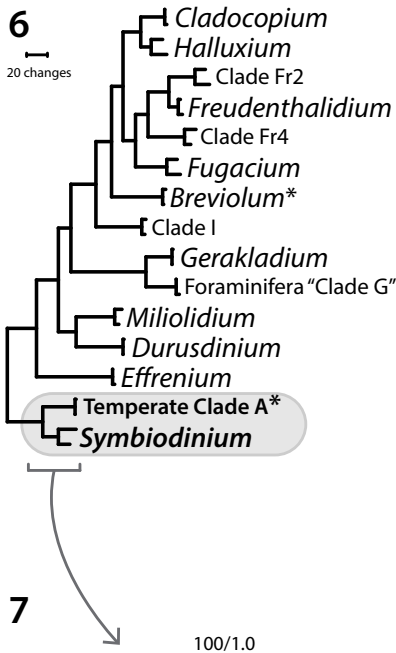
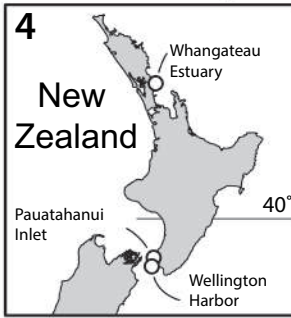
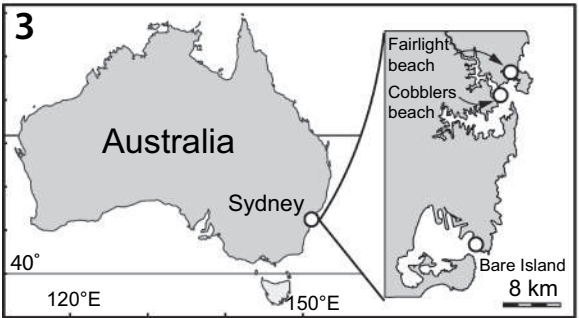
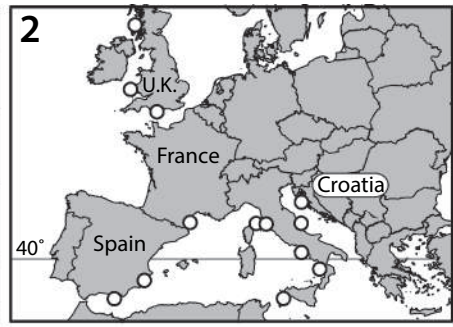
Holotype and neotype specimens, in their host tissues, were stored in a preservation buffer consisting of 20% DMSO, 25M EDTA, supersaturated with NaCl, developed by Seutin *et al.* (1991), placed in a 2 ml cryo-vial, and deposited at the US National Herbarium, National Museum of Natural History, Smithsonian Institution, Washington DC.

Results

Philozoon Geddes 1882 emend. LaJeunesse

Description

Single cells of the coccoid phase spherical to broadly ellipsoidal. Mean cell length 6.5–13.5 μm (Fig. 15). Associated with cnidarian hosts in coastal habitats from temperate zones in the northern and southern hemispheres. Species adapted to cold and seasonably variable environments. Dinoflagellates with partial sequences of LSU-rDNA (containing the variable domains D1/D2) differing ≤5% in nucleotide sequences from the type species *P. medusarum* (MW785231) are defined as members of this genus.



Figs 1–7. Collection locations in northern and southern hemispheres and phylogenetic analysis of *Philozoon*. **Fig. 1.** The distribution of *Philozoon* in northern and southern temperate zones. **Figs. 2–5.** Collection locations in the Mediterranean Sea and north-eastern Atlantic Ocean, Australia, New Zealand and Chile. **Fig. 6.** LSU rDNA based phylogeny of the family Symbiodiniaceae (modified from LaJeunesse *et al.*, 2018). Asterisks indicate symbionts from ‘temperate-A’ and *Breviolum* lineages known to associate with the rhizostome jellyfish *Cotylorhiza tuberculata*. **Fig. 7.** Phylogenetic reconstruction of the genus *Philozoon* based on concatenated sequences of nuclear ribosomal (LSU), chloroplast (cp23S) and mitochondrial (*cob* and *cox1*) genes. Outgroup species in the related genus *Symbiodinium* (formerly Clade A) were included for comparison. Phylogeny based on maximum parsimony and branch support assessed via bootstrapping and posterior probabilities.

GENUS ETYMOLOGY: From the Greek *phileo* meaning ‘to love as a friend’ and *zoon* meaning ‘animal’.

TYPE SPE CIE SP. *medusarum* Geddes emend. LaJeunesse & D’Ambra.

***Philozoon medusarum* Geddes 1882 emend. LaJeunesse & D’Ambra**

Description

Mean length of ovate coccoid cells 8.6–9.0 µm (Fig. 15). Nucleotide sequences of the LSU rDNA (GenBank MW785231), ITS rDNA (MW785239) and *psbA^{ncr}* (MW846168).

NEOTYPE: The neotype of *Philozoon medusarum* was deposited in the US National Herbarium, National Museum of Natural History, Smithsonian Institution, Washington DC, USA, as US Algal Collection specimen 227754.

NEOTYPE LOCALITY: Naples, Italy (40°01’25.3”N, 15°58’6”E). Collected by Isabella D’Ambra in August 2019.

***Philozoon actiniarum* Geddes 1882 emend. LaJeunesse, Casado-Amezúa & Wiedenmann (Fig. 8)**

Description

Mean length of ovate coccoid cells ranging from 6.5–9.2 µm (Fig. 15). Nucleotide sequences of the LSU rDNA (GenBank MW785232), ITS rDNA (MW785240), partial chloroplast large sub-unit, cp23S (MW785241), mitochondrial cytochrome b, *cob*, (MW819748), mitochondrial *cox1* (MW819741) and *psbA^{ncr}* (MW846169–MW846181) genetically define this species.

NEOTYPE: The neotype of *Philozoon actiniarum* was deposited in the US National Herbarium, National Museum of Natural History, Smithsonian Institution, Washington DC, USA, as US Algal Collection specimen 227755.

NEOTYPE LOCALITY: Calahonda, Granada, Spain (36°42’10.52”N, 3°24’40.71”W). Collected by Pilar Casado-Amezúa in August 2014.

HABITAT: Found as intracellular symbionts of the common sea anemone, *Anemonia viridis* Forsskål (1775), and all its ‘sub-species’ or ‘morpho-types’ living in shallow marine habitats in the Mediterranean Sea and in the north-eastern Atlantic (Mallien *et al.*, 2018).

ETYMOLOGY: Named after the cnidarian order for ‘true’ sea anemones, Actiniaria.

New species belonging to Philozoon

***Philozoon geddesianum* Wiedenmann & LaJeunesse, sp. nov. (Fig. 9)**

Description

Mean length of ovate coccoid cells 9.0–9.5 µm (Fig. 15). Nucleotide sequences of the large ribosomal subunit rDNA (GenBank MW785234), partial chloroplast large sub-unit, cp23S (MW785243), mitochondrial cytochrome b, *cob*, (MW819750), mitochondrial *cox 1* (MW819743) and *psbA^{ncr}* (MW846182–MW846185) genetically define this species.

HOLOTYPE: The holotype of *Philozoon geddesianum* sp. nov. was deposited in the US National Herbarium, National Museum of Natural History, Smithsonian Institution, Washington DC, USA, as US Algal Collection specimen 227757.

TYPE LOCALITY: Scotland, UK (56°57’27”N, 05°51’21”W). Collected by Joerg Wiedenmann in May 2019.

HABITAT: Found as intracellular symbionts of the sea anemone *Cereus pedunculatus* (Pennant, 1777), from shallow marine habitats in the eastern North Atlantic Ocean.

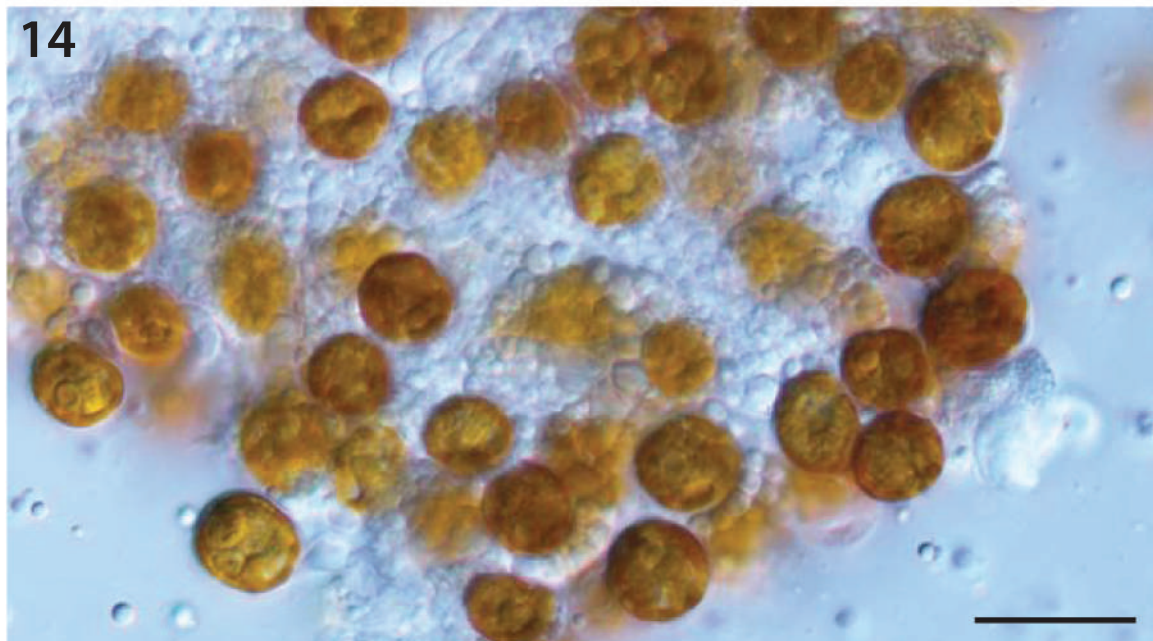
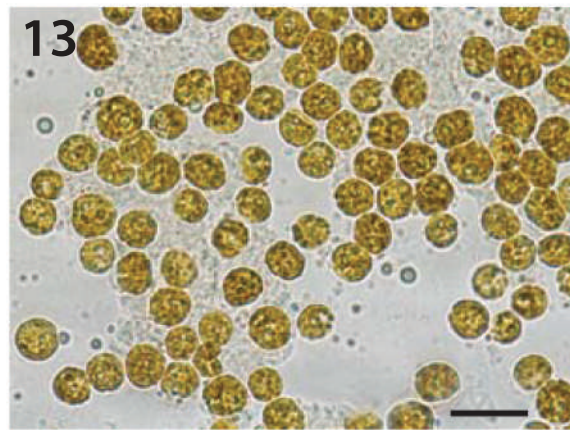
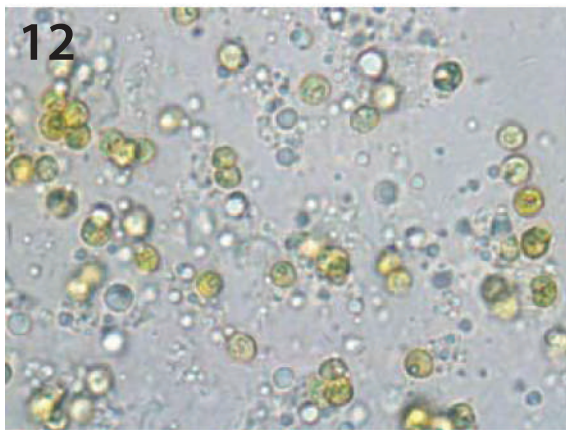
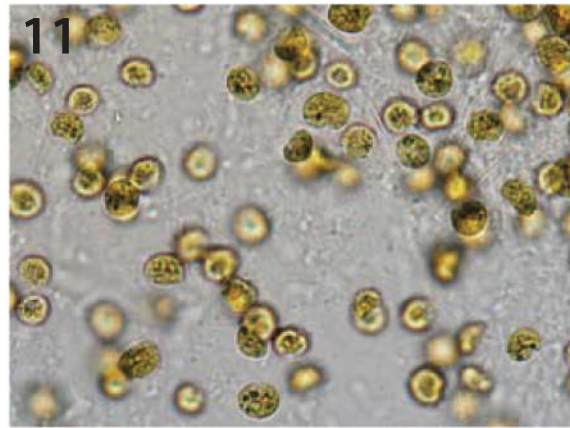
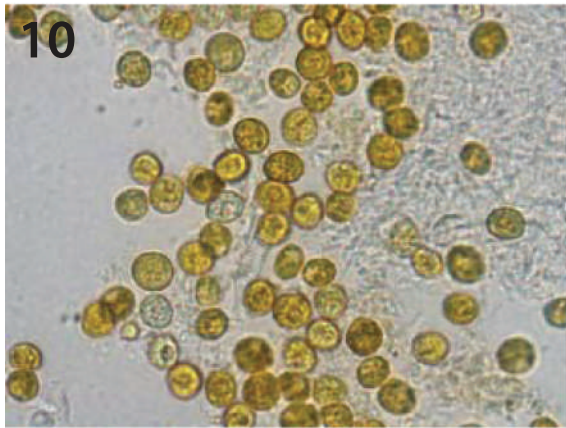
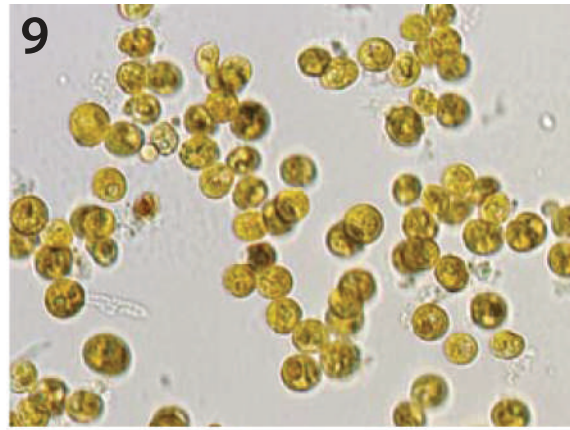
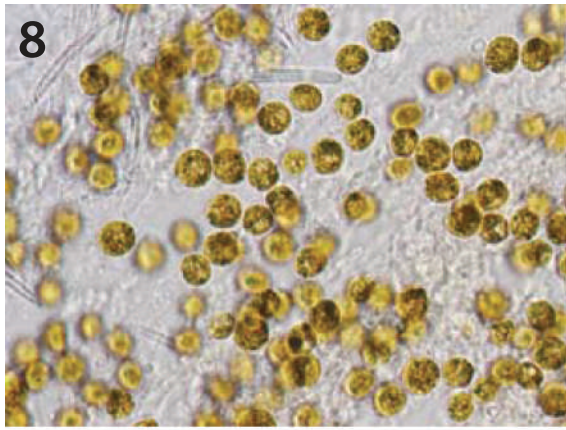
ETYMOLOGY: Named after the Scottish biologist Sir Patrick Geddes (1854–1932) who was among the first naturalists to recognize the existence of symbiotic algae benefiting animals and large protists. He was the first to argue that the ‘yellow cells’ were not parasites, but mutualistic symbionts because of their ubiquitous distribution in particular host taxa that were ecologically common relative to non-symbiotic animals (Geddes, 1882b).

***Philozoon adriaticum* LaJeunesse, Turnham & Wiedenmann, sp. nov. (Fig. 10)**

Description

Mean length of coccoid cells 9.6–10.8 µm (Fig. 15). Nucleotide sequences of the LSU rDNA (GenBank MW785238), partial chloroplast large sub-unit, cp23S (MW785247), mitochondrial cytochrome b, *cob*, (MW819754), mitochondrial cytochrome oxidase c subunit 1, *cox 1* (MW819747) and *psbA^{ncr}* (MW846203–MW846207) genetically define this species.

HOLOTYPE: The holotype of *Philozoon adriaticum* was deposited in the US National Herbarium, National Museum of Natural History, Smithsonian



Figs 8–14. Light micrographs of symbiont cells preserved from host tissues. Images (400×) representative of Fig. 8. *Philozoon actiniarum*, Fig. 9. *P. geddesianum*, Fig. 10. *P. adriaticum*, Fig. 11. *P. paranemonium*, Fig. 12. *P. balanophyllum*, Fig. 13. *P. anthopleurum* and Fig. 14. *P. colossum* (1000×). Scale bars = 20 μm.

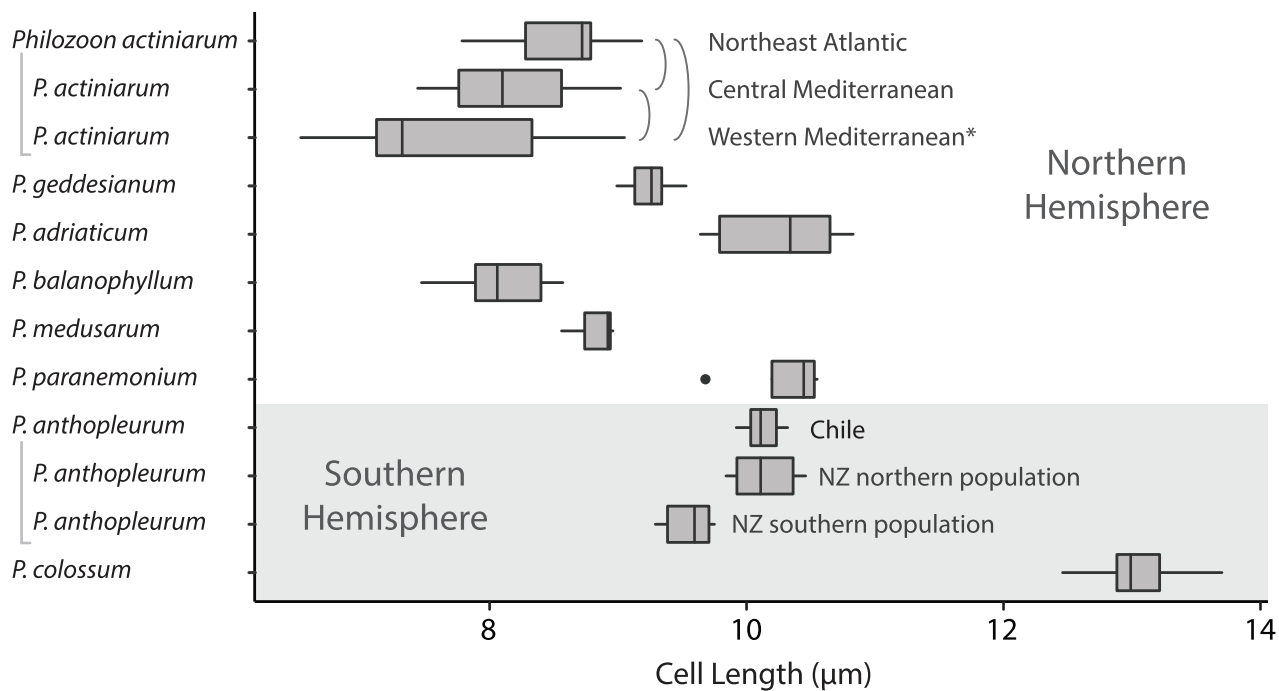


Fig. 15. Box plots identifying the mean, upper and lower quartiles and extremes of cell lengths among *Philozoon* spp. Data for each plot were obtained from 4–9 host specimens and based on measurements from 70–200 individual cells from each sample. Pairwise t-tests on *P. actiniarum* from three regions showed a statistically significant difference between North-eastern Atlantic and warmer less seasonal central Mediterranean.

Institution, Washington DC, USA, as US Algal Collection specimen 227761.

TYPE LOCALITY: Sv. Slatina, Njivice, Croatia (45° 09'04"N, 14°31' 32"E). Collected by Joerg Wiedenmann in August 2019.

HABITAT: Found as intracellular symbionts of the actinarian *Aiptasia mutabilis* from shallow marine habitats in the Mediterranean Sea.

ETYMOLOGY: Named after the Adriatic Sea where this species was first discovered.

***Philozoon paranemonium* LaJeunesse & Casado-Amezúa, sp. nov. (Fig. 11)**

Description

Mean length of ovate coccoid cells 9.7–10.6 µm (Fig. 15). Nucleotide sequences of the LSU rDNA (GenBank MW785233), partial chloroplast large sub-unit, cp23S (MW785242), mitochondrial cytochrome b, *cob*, (MW819749), mitochondrial *cox1* (MW819742) and *psbA^{ncr}* (MW846202) genetically define this species.

HOLOTYPE: The holotype of *Philozoon paranemonium* was deposited in the US National Herbarium, National Museum of Natural History, Smithsonian Institution, Washington DC, USA, as US Algal Collection specimen 227756.

TYPE LOCALITY: Isla del Ciervo, Murcia, Spain (37° 39'33.50"N, 0°44'7.23"W). Collected by Pilar Casado-Amezúa in September 2014.

HABITAT: Found as intracellular symbionts of the sea anemone, *Paranemonia cinerea* (Contarini), from shallow marine habitats in the Mediterranean Sea.

ETYMOLOGY: Named after the host genus, *Paranemonia*, which this endosymbiont inhabits.

***Philozoon balanophyllum* LaJeunesse, Wiedenmann & Goffredo, sp. nov. (Fig. 12)**

Description

Mean length of coccoid cells 7.7–8.4 µm (Fig. 15). Nucleotide sequences of the LSU rDNA (GenBank MW785236), partial chloroplast large sub-unit, cp23S (MW785245), mitochondrial cytochrome b, *cob*, (MW819752), mitochondrial *cox 1* (MW819745) and *psbA^{ncr}* (MW846186–MW846197) genetically define this species.

HOLOTYPE: The holotype of *Philozoon balanophyllum* was deposited in the US National Herbarium, National Museum of Natural History, Smithsonian Institution, Washington DC, USA, as US Algal Collection specimen 227759.

TYPE LOCALITY: Bagnara Calabria, Italy (38°15'N, 15° 49'E). Collected by Stefano Goffredo in April 2006.

HABITAT: Found as intracellular symbionts of the scleractinian, *Balanophyllia europaea* (Risso), from shallow marine habitats in the Mediterranean Sea.

ETYMOLOGY: Named after the host genus, *Balanophyllia*, which this endosymbiont inhabits.

***Philozoon anthopleurum* LaJeunesse, C.Oakley, S. Davy, Spano & Cubillos, sp. nov. (Fig. 13)**

Description

Mean length of coccoid cells 9.3–10.5 μm (SD \pm 0.2 μm ; Fig. 15). Nucleotide sequences of the LSU rDNA (GenBank MW785237), partial chloroplast large subunit, cp23S (MW785246), mitochondrial cytochrome b, *cob* (MW819753), mitochondrial *cox 1* (MW819746) and *psbA^{ncr}* (MW846208–MW846219) genetically define this species.

HOLOTYPE: The holotype of *Philozoon anthopleurum* was deposited in the US National Herbarium, National Museum of Natural History, Smithsonian Institution, Washington DC, USA, as US Algal Collection specimen 227760.

TYPE LOCALITY: Wellington Harbour, New Zealand (41°17'04.87"S, 174°47'33.36"E). Collected by Clint Oakley in 2017.

HABITAT: Found as intracellular symbionts of the sea anemone, *Anthopleura aureoradiata* Stuckey 1909 (= *A. hermaphroditica* Carlgren 1899; *sensu* Spano & Häussermann, 2017), from shallow marine habitats in New Zealand and Chile.

E TYMOLOGY: Named after the host genus, *Anthopleura*, which this endosymbiont inhabits.

NOTE S: *Anthopleura aureoradiata* is putatively synonymous with *A. hermaphroditica* Carlgren 1899 (*sensu* Spano & Häussermann, 2017).

***Philozoon colossus* LaJeunesse, M.Nitschke & Suggett, sp. nov. (Fig. 14)**

Description

Mean length of ovate coccoid cells 12.5–13.4 μm (Fig. 15). Nucleotide sequences of the LSU rDNA (GenBank MW785235), partial chloroplast large subunit, cp23S (MW785244), mitochondrial cytochrome b, *cob* (MW819751), mitochondrial *cox 1* (MW819744) and sequences of the *psbA^{ncr}* (MW846198–MW846201), genetically define this species.

HOLOTYPE: The holotype of *Philozoon colossus* was deposited in the US National Herbarium, National Museum of Natural History, Smithsonian Institution, Washington DC, USA, as US Algal Collection specimen 227758.

TYPE LOCALITY: Sydney Harbour, Australia (33°49' 31.31"S, 151°15' 46.25"E). Collected by Matthew R. Nitschke in 2015.

HABITAT: Found primarily as intracellular symbionts of the alcyonacean soft coral, *Capnella gaboensis* (Verseveldt), collected from shallow marine habitats in the temperate zone of south-eastern Australia.

ETYMOLOGY: From the Latin *colossus* meaning 'gigantic'.

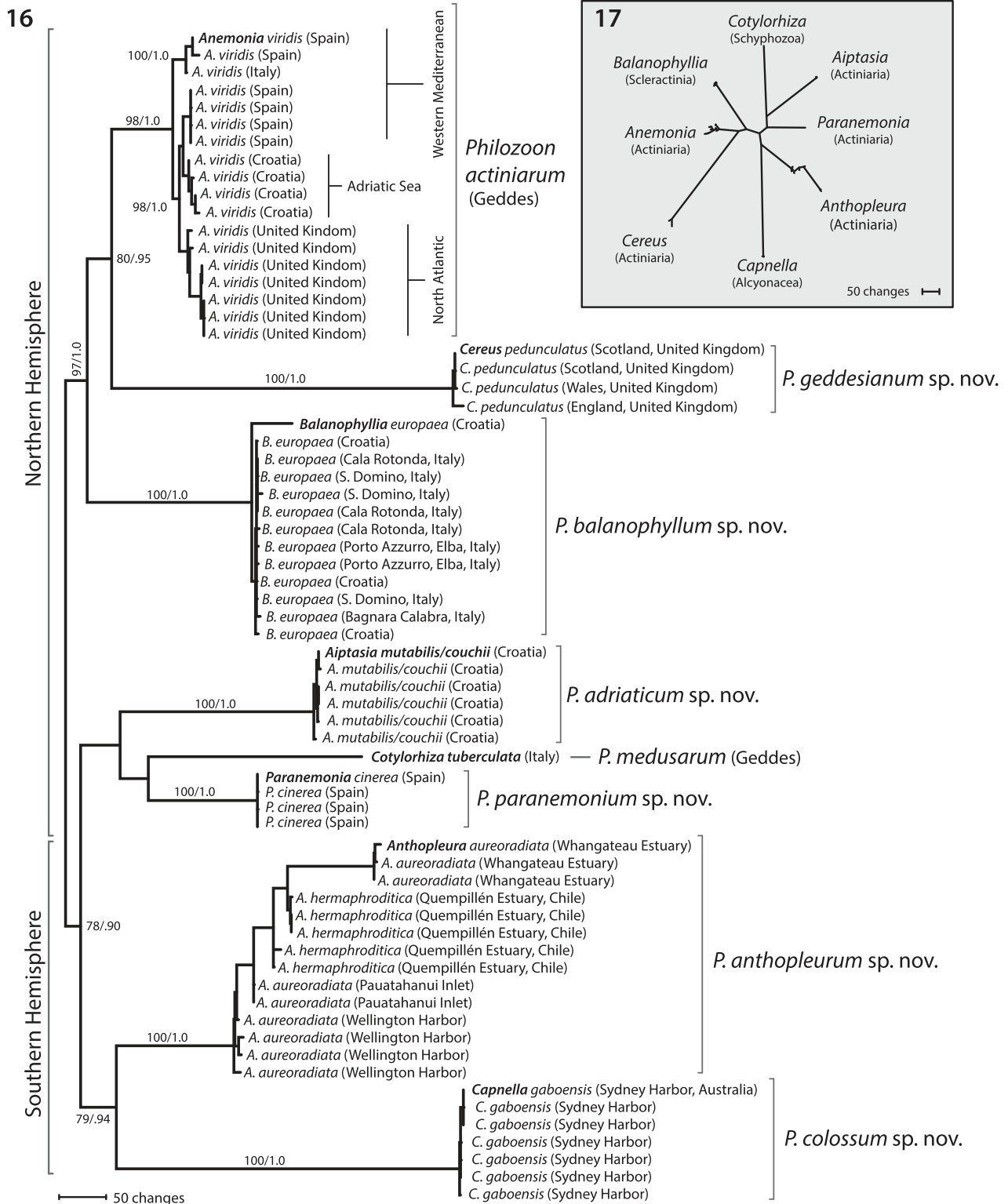
Broad range in cell sizes among species of Philozoon

Mean lengths of ovate coccoid cells of *Philozoon* species ranged from \sim 6.5 (\pm 0.6) μm among genotypes of *P. actiniarum* to mean cell lengths reaching \sim 13.4 (\pm 1.2) μm among genotypes of *P. colossus* (Figs 8–15). While there was variation in cell sizes (Fig. 15), mean size ranges among individual samples (based on 75–200 cells per sample) differentiated large, medium and small species of *Philozoon*. *Philozoon actiniarum* exhibited the greatest variation in cell size (Fig. 15), while *P. colossus* from the soft coral *Capnella gaboensis* was the largest with mean cell lengths substantially larger ($>$ 2.0 μm) than other *Philozoon* species.

Delimiting species of Philozoon with genetic evidence

The sequence analysis of LSU rDNA from specimens of *Cotylorhiza tuberculata* (= *Cassiopeia borbonica*) assigned its symbionts to the temperate-A lineage as well as *Breviolum* sp. (Fig. 6). These two symbionts differed in relative concentrations depending on the individual host analysed (Mordret & D'Ambra, unpubl.). The combined phylogenetic analyses of conserved nuclear ribosomal (LSU rDNA), chloroplast (cp23S) and mitochondrial genes (*cob* and *cox 1*) resolved multiple new *Philozoon* species (Fig. 7; Supplementary data S1). However, when analysed independently, sequence comparisons for a given gene differentiated subsets of these species. Certain markers did not resolve certain species, but never contradicted one another. For a given gene, all examined specimens of a species possessed the same sequence variant. LSU resolved *P. colossus* and *P. adriaticum*, but was identical for *P. medusarum*, *P. actiniarum* and *P. geddesianum*, and identical for *P. paranemonium*, *P. balanophyllum* and *P. anthopleurum*. The chloroplast 23S resolved both *P. colossus* and *P. geddesianum* but was unable to resolve the other species. The *cox1* resolved *P. colossus*, *P. geddesianum*, *P. balanophyllum*, *P. paranemonium*, but did not separate *P. actiniarum* from *P. anthopleurum*, while *cob* resolved *P. adriaticum* from all others in the genus.

The nucleotide sequences of the *psbA* non-coding region (*psbA^{ncr}*) resolved each species lineage by large sequence differences (Fig. 16; Supplementary data S2). The broadly sampled *psbA^{ncr}* haplotypes of *P. actiniarum* exhibited the greatest amount of sequence variation and revealed phylogeographic patterning corresponding to distinct regions where specimens were collected (Fig. 16). The length (nt) of *psbA^{ncr}* differed consistently among species. It was largest for *P. geddesianum* (\sim 1680–1750 bp) and *P. balanophyllum* (\sim 1200 bp) and smallest for *P. anthopleurum* (\sim 825–950 bp).



Figs 16–17. Phylogenetic relationships among *Philozoon* spp. based on the rapidly evolving *psbA^{ncr}*. **Fig. 16.** Phylogenetic reconstruction of *Philozoon* spp. based on maximum parsimony of the partial coding and full non-coding regions of the *psbA* mini-circle gene. Branch support assessed via bootstrapping and posterior probabilities. **Fig. 17.** Redrawn *psbA^{ncr}* phylogeny illustrating the adaptive radiation of *Philozoon* and diversification corresponding to ecological specialization on Actiniaria, Scleractinia, and Alcyonacea hosts from temperate zones.

Genetic analyses of specimens of *Cotylorhiza tuberculata* from around Naples, Italy, the approximate locality where Geddes conducted his experiments, identified the presence of two symbiodiniacean species. The

co-occurrence of *Breviolum psygmophilum* LaJeunesse, J.E.Parkinson & J.D.Reimer in samples prevented sequencing of chloroplast cp23S and the mitochondrial *cox1* and *cob* genes for *Philozoon medusarum*.

Ecologically differentiated Philozoon spp.

Based on samples collected for the purposes of this research, members of *Philozoon* were found in sea anemones (Order Actiniaria), soft corals (Octocorallia: Order Alcyonacea), stony corals (Order Scleractinia) and scyphomedusae (Order Rhizostomeae). Each species of *Philozoon* was consistently identified from the same host species sampled multiple times and, in most cases, over multiple locations from different years (Supplementary table S1). For example, individuals of *Anemonia viridis* obtained from distant localities in the Western and Central Mediterranean as well as the North East Atlantic harboured only *P. actiniarum* (Fig. 16). A distinctive lineage observed from two samples of *Paranemonia* sp. from Spain indicated the likely existence of additional *Philozoon* species (data not shown).

Discussion

While Patrick Geddes was ahead of his time in thinking about the biology and ecology of the organisms he studied (see below), he provided minimal details for his new genus and species. The symbionts he designated as *Philozoon radiolarum* in radiolarian protists and *P. siphonophorum* in the free-floating hydrozoan *Velevella velevella* Linnaeus, are in the order Peridiniales and assigned to the genus *Zooxanthella* (Gast & Caron, 1996; Gottschling & McLean, 2013; Probert *et al.*, 2014; Yuasa *et al.*, 2016). However, as revealed by this work, his species, *P. medusarum*, associated with the jellyfish *Cotylorhiza tuberculata* (= *Cassiopea borbonica*) and *P. actiniarum*, assigned to symbionts in the sea anemone *Anemonia viridis*, are members of the family Symbiodiniaceae. Hence *Philozoon* is resurrected here to fill an important gap in the family's systematics and taxonomy, and to recognize the brief but important contributions of Geddes.

As the most dominant group of symbiotic dinoflagellates, the Symbiodiniaceae occur mainly in hosts from warm, seasonably stable, tropical and sub-tropical settings (LaJeunesse, 2020). However, members of the genus *Philozoon* exemplify how some have expanded geographically to become adapted to cold-water, seasonally variable, temperate zone environments (Fig. 1). The phylogeny based on conserved gene sequences, showing lineages differentiated by fixed albeit small differences, corresponds to the deep branching phylogeny of the *psbA^{ncf}*. This non-coding region evolves at rates 20–30 times faster than ITS rDNA and better emphasizes the extent to which species lineages of *Philozoon* have diverged from each other (Fig. 16; LaJeunesse & Thornhill, 2011). Substantiated by population genetic data in other studies, this marker provides a reliable proxy for delineating closely related species (LaJeunesse *et al.*, 2014; Thornhill *et al.*, 2014; Ramsby *et al.*, 2017; Wham *et al.*, 2017; Lewis *et al.*, 2019; Turnham *et al.*, 2021).

Adaptations to temperate zone environments

It is realistic to assume that *Philozoon* spp. have adaptations that enable them to cope with broad seasonal changes in temperature and light as well as enhanced abilities to acclimate to shorter and pronounced thermal fluctuations from upwelling events and tidal cycles. Temperate zones are subject to marked seasonal changes in temperature, irradiance and nutrients (Muller-Parker & Davy, 2001). At their latitudinal extremes (~50°N and 46°S), hosts mutualistic with *Philozoon* experience water temperatures that may reach winter lows of 6–8°C. During low tides, some partnerships can endure temporary exposure to even freezing air temperatures (Howe, 2013). Summer temperatures in these high latitudes reach 20–30°C, constituting an annual shift ≥10–15°C (e.g. Cubillos *et al.*, 2018).

Adaptation to high latitude environments may condition *Philozoon* spp. to tolerating future increases in atmospheric CO₂. Carbon dioxide concentrations are greater and more variable in cold-water temperate environments. Observations from natural CO₂ vent sites in the Mediterranean demonstrated that *Philozoon actiniarum* (designated ITS2 type A19 in Suggett *et al.*, 2012) in *Anemonia viridis* was unaffected by dynamic CO₂ environments, and even exhibited higher photosynthetic rates under raised CO₂ concentrations (Suggett *et al.*, 2012). Future studies assessing the generality of enhanced photosynthesis under high CO₂ tensions among *Philozoon* spp. may reveal the underlying mechanisms increasing photosynthesis and how such physiological processes compare with other Symbiodiniaceae.

Large quantities of particulate food are available in most temperate marine ecosystems. In contrast to tropical oligotrophic waters, high phytoplankton densities and greater turbidity in nutrient-rich temperate zone waters can diminish access to sufficient light. The availability of bountiful particulate matter in light-limited conditions may not favour the ecological persistence or success of dinoflagellate-cnidarian mutualisms and might explain the smaller number of symbiotic species in temperate habitats compared with tropical conditions. For instance, only five cnidarian species from the British Isles (NE Atlantic) are symbiotic (Muller-Parker & Davy, 2001). However, the oceanography of the Mediterranean Sea creates high irradiance oligotrophic environments. This may explain why many of the host species (~12 cnidarians) in the Mediterranean are symbiotic, and most of these with *Philozoon* (Casado-Amezúa *et al.*, 2016; Visram *et al.*, 2006).

For hosts populated with *Philozoon*, autotrophy may account for a portion of their metabolic needs (Farrant *et al.*, 1987; Davy *et al.*, 1996, 1997; Gibbons, 2008). In combination with heterotrophy, the additional source of energy from photosynthesis may be especially important during nutritionally poor conditions of late spring

and summer (Farrant *et al.*, 1987; Cocito *et al.*, 2013). This advantage may partially explain why temperate anthozoans harbouring *Philozoon* are particularly abundant. *Anemonia viridis*, for instance, is one of the most common sea anemones in the north-eastern Atlantic and Mediterranean Sea (Geddes, 1882b; Muller-Parker & Davy, 2001) and can proliferate on suitable shallow water substrates via asexual reproduction (Wiedenmann *et al.*, 2007).

Symbiotic cnidarians with *Philozoon* occur in many temperate provinces (Fig. 1), but not all, apparently. In well-studied regions like the west (Secord & Augustine, 2000) and east (Thornhill *et al.*, 2008) coasts of North America, symbionts are represented by dinoflagellates in the genus *Breviolum* (formerly Clade B). *Cladocopium* (formerly Clade C) is the most prevalent symbiont group in temperate symbioses from the north-west Pacific (Lien *et al.*, 2012) and the south-west coast of Australia (Silverstein *et al.*, 2011). Sampling from the Atlantic and Pacific coasts of temperate Africa and temperate east coast of South America is needed to determine whether *Philozoon* occurs in symbiotic animals from these regions.

The biogeography and evolution of Philozoon spp.

The present distribution and diversity of *Philozoon* likely emerged in response to reductions in global temperatures that started during the late Miocene/early Pliocene, and then intensified during the Pleistocene (Zachos *et al.*, 2008). This precipitous cooling and subsequent oscillation between warmer and colder periods of Earth's climate has generated genetic evidence that provides clues to explain the current antitropical 'bipolar' distribution of *Philozoon* (Stepanjants *et al.*, 2006). With the onset of northern hemisphere glaciation (~2.7 mya) major oceanic cold-water frontal systems migrated toward and retreated from the equator 40 or more times. Because species of *Philozoon* can tolerate tropical temperatures (e.g. Bythell *et al.*, 1997; Ferrier-Pagès *et al.*, 2009), the cooler and compressed tropics of glacial intervals may not have posed a significant biogeographic barrier to dispersal. Moreover, the genetic similarity of *P. anthopleurum* in New Zealand and Chile shows that these microalgae undergo pulses of long-distance dispersal and that the host *Anthopleura* from each region may also be genetically connected (Fig. 16). During one of these early glaciations, an ancestral progenitor successfully spread to hosts in both temperate zones. Once compatibility with hosts in each region was achieved, ecologically separate populations began to diversify (Fig. 17).

The major shifts in climate during the Pliocene and Pleistocene appear to have initiated independent adaptive radiations in multiple symbiodiniacean

genera (LaJeunesse *et al.*, 2010; Thornhill *et al.*, 2014; Lewis *et al.*, 2019). Study of the phylogeny of *Philozoon* hints at a sequence of events that led to the present-day diversity of the genus. Its polytomy signifies that diversity in the genus is also a product of a recent adaptive radiation (Fig. 17), and that host (ecological) specialization was the major factor that influenced the evolution of each species (LaJeunesse, 2005; LaJeunesse *et al.*, 2014; Thornhill *et al.*, 2014; Lewis *et al.*, 2019).

The high host fidelity possessed by each *Philozoon* species is a significant ecological attribute that emerges from selection pressures promoting specific resource use. Indeed, ecological specialization is a common process that creates genetic isolation and ultimately leads to speciation (Hendry *et al.*, 2007; Schluter & Conte, 2009). In these cases, the broad taxonomic disparity of hosts including different species of sea anemone, a stony coral, a soft coral and a scyphozoan jellyfish all represent unique habitat resources. Symbiont speciation is especially enhanced between populations associated with host taxa that obtain symbionts from their parents (Barneah *et al.*, 2004; LaJeunesse *et al.*, 2004; Thornhill *et al.*, 2014). Having a closed mode of symbiont acquisition (i.e. vertical transmission) is characteristic of many, but not all, temperate hosts (Muller-Parker & Davy, 2001).

Patrick Geddes's contributions to our understanding of algal symbionts

Sir Patrick Geddes (1854–1932) had a productive and varied career in the disciplines of biology, sociology, geography, urban planning, education and philanthropy (Fig. 18; Stalley, 1972). While a lecturer at Edinburgh University (1880–1888), he published consequential observations and thoughts on the physiology and ecology of animals containing chlorophyll based on investigations carried out at the Stazione Zoologica Anton Dohrn in Naples, Italy. At this time, he belonged to a small group of naturalists, including Leon Cienkowski, Geza Enz, Richard and Oscar Hertwig and Karl Brandt, who endeavoured to determine the identities and relationships of tiny green and yellow cells found in certain marine and freshwater organisms.

Geddes's insight on the nature of yellow cells in animals and protists published in *Nature* (26 January 1882a: 303–305) surpassed that of his contemporaries who generally regarded these cells as parasites. Brandt (1881) was the first to show definitively, by identifying their nuclei, that these cells were 'morphologically independent unicellular organisms', and also showed experimentally that these 'zooxanthellae' helped to 'sustain' their hosts, but never expanded on these observations (Krueger, 2017). However, it was Geddes (1882b) who unequivocally recognized the

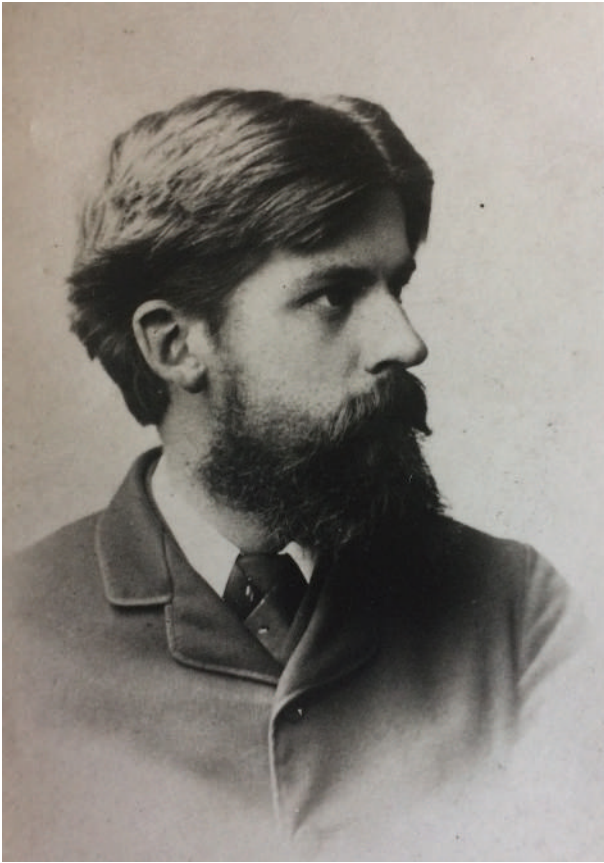


Fig. 18. Portrait of Sir Patrick Geddes (c. 1888) at the age of 34, several years after publishing observations from his experiments on animals containing chlorophyll. (Image courtesy of libraryblogs.is.ed.ac.uk).

full significance of the evidence before him. In ascribing the associations as *animal lichens*, he declared eloquently: ‘Such an association is far more complex than that of the fungus and alga in the lichen, and indeed stands unique in the physiology as the highest development, not of parasitism, but of the reciprocity between the animal and vegetable kingdoms.’ Geddes vigorously contended that these algae were mutualistic in nature and deduced this using a line of reasoning reintroduced nearly 100 years later to describe the mutualistic benefits of the partnership (e.g. Muscatine, 1974).

‘Thus, then, for the vegetable cell no more ideal existence can be imagined than that within the body of an animal cell of sufficient active vitality to manure it with carbonic acid (=CO₂) and nitrogen waste, yet of sufficient transparency to allow the free entrance of the necessary light. And conversely, for an animal cell there can be no more ideal existence than to contain a vegetable cell, constantly removing its waste products supplying it with oxygen and starch ...’ (Geddes, 1882b: 304)

Moreover, Geddes also emphasized that animals and radiolarians with yellow cells were far more abundant in the habitats where he collected specimens compared with non-symbiotic species.

Therefore, not only did he recognize the significance of a physiologically coupled host and symbiont, he also first highlighted the ecological implications of these mutualisms being dominant in nature when and where they occurred.

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Supplementary information

The following supplementary material is accessible via the Supplementary Content tab on the article’s online page at <http://dx.doi.org/10.1080/09670262.2021.1914863>:

Supplemental table S1. Sample metadata including host species, geographic locality, depth of collection and latitude–longitude coordinates.

Supplemental data S1. Nexus file containing the alignment of concatenated LSU, cp23S, *cob* and *cox1* sequences corresponding to species of *Philozoan* and *Symbiodinium* and used to create Fig. 7.

Supplemental data S2. Nexus file containing the alignment of *psbA*^{ncr} sequences representative of each *Philozoan* spp. used to create Figs 16 and 17.

Author contributions

T. C. LaJeunesse: original concept, conducted genetic analyses, microscopy, morphological analysis, created figures, led drafting and editing manuscript; J. Wiedenmann: original concept, sampling, drafting and editing manuscript; P. Casado-amezúa: original concept, sampling, drafting and editing manuscript; I. D'Ambrá: sampling and editing manuscript; K. E. Turnham: microscopy and editing manuscript; M. R. Nitschke: sampling, microscopy, morphological analysis, editing manuscript; C. A. Oakley: sampling and editing manuscript; S. Goffredo: sampling and editing manuscript; C. A. Spano: sampling and editing manuscript; V. M. Cubillos: sampling and editing manuscript; S. K. Davy: sampling and editing manuscript; D. J. Suggett: sampling and editing manuscript.

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